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ORIGINAL REPORT

Increased Risk for Other Cancers in Addition to Breast Cancer for CHEK2*1100delC Heterozygotes Estimated From the Copenhagen General Population Study

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ABSTRACT

Purpose

CHEK2 is a cell cycle checkpoint regulator, and the CHEK2*1100delC germline mutation leads to loss of function and increased breast cancer risk. It seems plausible that this mutation could also predispose to other cancers. Therefore, we tested the hypothesis that CHEK2*1100delC heterozygosity is associated with increased risk for other cancers in addition to breast cancer in the general population.

Patients and Methods

We examined 86,975 individuals from the Copenhagen General Population Study, recruited from 2003 through 2010. The participants completed a questionnaire on health and lifestyle, were examined physically, had blood drawn for DNA extraction, were tested for presence of CHEK2*1100delC using Taqman assays and sequencing, and were linked over 1943 through 2011 to the Danish Cancer Registry. Incidences and risks of individual cancer types, including breast cancer, were calculated using Kaplan-Meier estimates, Fine and Gray competing-risks regressions, and stratified analyses with interaction tests.

Results

Among 86,975 individuals, 670 (0.8%) were CHEK2*1100delC heterozygous, 2,442 developed breast cancer, and 6,635 developed other cancers. The age- and sex-adjusted hazard ratio for CHEK2*1100delC heterozygotes compared with noncarriers was 2.08 (95% CI, 1.51 to 2.85) for breast cancer and 1.45 (95% CI, 1.15 to 1.82) for other cancers. When stratifying for sex, the age-adjusted hazard ratios for other cancers were 1.54 (95% CI, 1.08 to 2.18) for women and 1.37 (95% CI, 1.01 to 1.85) for men (sex difference: P = .63). For CHEK2*1100delC heterozygotes compared with noncarriers, the age- and sex-adjusted hazard ratios were 5.76 (95% CI, 2.12 to 15.6) for stomach cancer, 3.61 (95% CI, 1.33 to 9.79) for kidney cancer, 3.45 (95% CI, 1.09 to 10.9) for sarcoma, and 1.60 (95% CI, 1.00 to 2.56) for prostate cancer.

Conclusion

CHEK2*1100delC heterozygosity is associated with 15% to 82% increased risk for at least some cancers in addition to breast cancer. This information may be useful in clinical counseling of patients with this loss-of-function mutation.

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INTRODUCTION

Cancer is a leading cause of death worldwide, accounting for 8.2 million deaths in 2012.[1] In cancer cells, normal control mechanisms of the cell cycle are defective, leading to uncontrolled growth.[2] The CHEK2 protein is a cell cycle checkpoint regulator activated by DNA damage, forcing the cell to either apoptosis or to cell cycle arrest until the DNA is repaired.[3-5] The CHEK2*1100delC germline loss-of-function mutation is a deletion of a single nucleotide, and the resulting frameshift abrogates the kinase function, which leads to genomic instability and an increased mutation rate.[6,7]

CHEK2*1100delC was first found in 1999 in Li-Fraumeni-like families[8] and the mutation is now an established breast cancer susceptibility allele.[7,9-14] In the Netherlands, the CHEK2*1100delC carrier frequency is 1.2% (95% CI, 0.7% to 1.8%),[15] the highest known frequency in northern and western Europe. CHEK2*1100delC carrier status is associated with a two- to three-fold increased risk for breast cancer in the general population at

a median age of 53 years,[7] and sisters and mothers of female CHEK2*1100delC carriers with breast cancer, who themselves are mutation heterozygotes, have cumulative incidences of 85% for breast cancer at an average age of 70 years, and of 37% at an average age of 49 years,[16] due to having both the mutation and an affected first-degree relative, and they are thus eligible for breast cancer surveillance programs.[16-18] Therefore, genotyping for CHEK2*1100delC is part of the Dutch genetic screening for families with breast cancer.[19] Considering the crucial function of CHEK2 for cell cycle control, one would expect this mutation to be associated with cancer overall and not only breast cancer. However, studies focusing on other types of cancer have provided conflicting results.[7,20-26]

Male CHEK2*1100delC heterozygotes have increased risk for prostate cancer,[7,21,22] and particularly for familial prostate cancer.[27] CHEK2*1100delC may also be an important colorectal cancer-predisposing gene, although the increased risk seems to be primarily in the familial forms.[7,20,25] Few studies have investigated malignant melanoma, kidney cancer, thyroid cancer, brain cancer, lung cancer, esophageal cancer, ovarian cancer, and leukemia.[7,15,23,26,28-31] More and larger studies are needed to confirm or rebut these results. We hypothesized that CHEK2*1100delC heterozygosity is associated with increased risk for other cancers in addition to breast cancer in the general population. To test this hypothesis, we genotyped 86,975 individuals from the Copenhagen General Population Study.

PATIENTS AND METHODS

Study Design

Settings and participants. The Copenhagen General Population Study was initiated in 2003 with ongoing enrollment.[32] White participants and those of Danish descent from certain areas of Copenhagen were randomly invited, using the Danish Civil Registration System, with a participation fraction of 44%. Participants completed a self-administered questionnaire concerning lifestyle and health status, which was reviewed with an investigator on the day of attendance. A physical examination was performed and blood samples for biochemical analyses and DNA extraction were drawn. Date of death (n = 4,012) and emigration (n = 294) were determined from the Danish Civil Registration System, which led to censoring in statistical analyses of cancer risk. We included 86,975 participants, with no overlap of participants from our previous study on the basis of the Copenhagen City Heart Study.[7]

All participants gave written informed consent. The study was approved by the Herlev and Gentofte Hospital and by a Danish ethical committee (H-KF-01-144/01), and was conducted according to the Declaration of Helsinki.

Cancer end points. Since 1943, cancer diagnoses in Denmark have been registered in the national Danish Cancer Registry[33] and, since 1987, this has been compulsory for all physicians by law.[34] Cancer diagnoses and dates were collected by linking each participant's unique Civil Registration Number to the national Danish Cancer Registry, which records approximately 98% of all cancers in Denmark.[33] Diagnoses of invasive cancer were made using the seventh or tenth editions of the WHO International Classification of Diseases.[35,36]

The end point for other cancers in addition to breast cancer was defined as all types of cancer combined, except breast and nonmelanoma skin cancer. We found no heterozygotes with laryngeal cancer, pancreatic cancer, liver cancer, non-Hodgkin's lymphoma, and esophageal cancer, and, therefore, could not estimate risk for these cancers.

Covariates. Information on smoking habits was obtained from the self-reported questionnaires and former and current smokers were categorized as ever-smokers. For ever-smokers, we calculated the cumulative tobacco consumption in pack-years, where 1 pack-year was defined as consumption of 20 cigarettes or the equivalent per day for 1 year. Participants were asked about the type and units of weekly alcohol consumption, which was converted to grams per week; one unit is approximately 12 g of alcohol. When stratifying for alcohol consumption, high alcohol consumption was defined according to the Danish Health and Medicines Authority as more than 168 g/wk for men and 84 g/wk for women. Participants were dichotomized according to exercise level, and low physical activity was defined as predominantly sitting work and fewer than 4 hours of light physical activity in leisure time. High meat intake was defined as eating beef, veal, or pork more than four times a week. Low educational level was fewer than 10 years of school education. Low income was less than 200,000 Danish Kroner yearly, corresponding to approximately €27,000. The number of first-degree relatives was obtained from the Danish Civil Registration System, and their cancer diagnoses were collected from the national Danish Cancer Registry. Only individuals with one or more first-degree relatives (n = 81,162) were included in the particular analysis. On the basis of this information, we categorized the participants into the following groups: with or without family history of breast cancer, and with or without family history of other cancers. Premenopausal women reported use of oral contraceptives and postmenopausal women reported use of hormone replacement therapy. Women also reported the number of children they had and their age when their first child was born. Body mass index was calculated as measured weight divided by measured height squared. Plasma C-reactive protein was measured and dichotomized in two categories, with a high level defined as above 3 mg/L, as done previously.[37,38]

We lacked information on 0.8% of covariates, except for first-degree relative cancer status, for which we lacked information on 6.7% of individuals.

Genotyping. Leukocyte DNA was extracted from peripheral blood, using Qiagen blood kits (Qiagen, Hilden, Germany).[39] The CHEK2*1100delC mutation was detected with a polymerase chain reaction-based Taqman assay (Applied Biosystems, Waltham, MA), as described previously.[13] All participants were genotyped using identical procedures in the same laboratory, and each run included positive and negative controls. All presumed heterozygous samples were sequenced and individuals were only categorized as heterozygotes if both assays demonstrated the CHEK2*1100delC mutation. For failed samples, the analysis was repeated, leading to a call fraction of 99.7%; the 261 individuals without a genotype call were not included. The observed allele frequency of CHEK2*1100delC was 0.385%. In the simulation, the two alleles per genotype were randomly and independently assigned to 86,975 artificial individuals, and each allele with a probability of 0.385% was to be CHEK2*1100delC. The two alleles were combined into a genotype, and genotype frequencies from 10,000 runs were collapsed into one dataset. The obtained normal distribution of CHEK2*1100delC homozygotes was compared with the observed number (n = 0).

Statistical Analyses

Statistical analyses were carried out using STATA 13.1 SE software (StataCorp, College Station, TX). A two-sided P value less than .05 was considered statistically significant. Wilcoxon or χ^2 tests were used to compare characteristics in CHEK2*1100delC heterozygotes and non-carriers. When appropriate, we also adjusted for multiple tests according to the Bonferroni method. We used a Fine and Gray competing risk-regression model,[40] with age as the time scale to estimate hazard ratios with 95% CIs, thus automatically adjusting for age and referred to as age adjusted. Hazard ratios from this type of regression modeling are also known as subhazard ratios. The primary model was also adjusted for sex. To adjust for other possible confounders of cancer risk, we used two different multivariable models. One also included smoking in pack-years, alcohol consumption, meat intake, physical activity, income, education, family history of breast cancer, family history of other cancers, body mass index, and plasma C-reactive protein. The other model further included potential confounders specifically for female cancers (i.e., nulliparity, numbers of children, use of oral contraceptives, and hormone replacement

therapy). The latter model was used for breast cancer, uterus cancer, ovary cancer, and cervix cancer. The assumption of proportional hazards was assessed graphically and by using Schoenfeld residuals, and we did not detect major violations. Death and emigration were used as competing events. Because genotype does not change through life, and to ignore pediatric cancer types, we followed all individuals from the participant's 20th year birthday. The model allowed for follow-up before and after blood sampling as a time-varying covariate. Thus, typically, we followed an individual from his or her 20th birthday until time of blood sampling (i.e., follow-up period before blood sample), and from time of blood sampling until end of follow-up (i.e., follow-up period after blood sample). If we did not take the follow-up period into account, results were similar, however. The follow-up period ended at the date of the cancer diagnosis in question, death (n = 4,012), emigration (n = 294), or at December 31, 2011 (last date with complete information from the Danish Cancer Registry), whichever came first. The median follow-up time was 42 years (range, 28 days to 69 years), and did not differ by genotype ($P = .43$). In addition to Fine and Gray competing risk-regression models, we also estimated risk on the basis of Cox proportional hazard regression models, with identical covariates for adjustment, as in the Fine and Gray models.

The null hypothesis of no association between *CHEK2*1100delC genotype and risk for any of the 19 cancer types was examined by testing whether the distribution of the 19 P values deviated from the uniform distribution. The sum of $-2 \times \ln(P \text{ value})$ was calculated and compared with the χ^2 distribution with 38 degrees of freedom.[41] The distribution of observed P values of the cancers was compared in a quantile-quantile plot to the expected (uniform) distribution of P values assuming no association.

RESULTS

Among 86,975 participants from the Copenhagen General Population Study, 670 (0.8%) were *CHEK2*1100delC heterozygotes and 86,305 (99.2%) were noncarriers (Table 1). No homozygotes were identified. This distribution did not differ from Hardy-Weinberg equilibrium ($P = .25$). Given the 670 heterozygotes detected, the expected number of *CHEK2*1100delC homozygotes was 1.3 (95% CI, 0.0 to 3.5; 10,000 rounds of genotype simulations). There were no major differences between heterozygotes and noncarriers for any of the established cancer risk factors and, thus, potential confounders (Table 1), although being nulliparous, and having a family history, was more common among heterozygotes than among noncarriers.

Incidence and Risk for Breast Cancer and Other Cancers

Of all women, 2,442 developed breast cancer, and of all participants, 6,635 developed other cancers. *CHEK2*1100delC heterozygotes compared with noncarriers had increased incidence of other cancers (log-rank $P = .001$). From a model taking competing events of death and emigration into consideration, the age- and sex-adjusted hazard ratio was 1.45 (95% CI, 1.15 to 1.82; Fig 1C). *CHEK2*1100delC heterozygotes compared with noncarriers had increased incidence of breast cancer (log-rank $P = .001$). From a model taking competing events of death and emigration into consideration, the age-adjusted hazard ratio was 2.08 (95% CI, 1.51 to 2.85; Fig 1D). Results from Cox proportional hazard regression models were not different (Figs 1A and 1B).

Risk for Other Cancers by Strata of Cancer Risk Factors

Risk for other cancers for *CHEK2*1100delC heterozygotes was not different when participants were stratified according to sex, age, smoking status, alcohol consumption, nulliparity, family history of breast cancer and other cancers, body mass index, plasma C-reactive protein, and follow-up period before and after

Table 1. Characteristics of Participants From the Copenhagen General Population Study by Genotype, Recorded at the Day of Examination

Characteristics	CHEK2*1100delC Noncarriers	CHEK2*1100delC Heterozygotes	P*
No. (%)	86,305 (99.2)	670 (0.8)	
Women	55	57	.37
Age, years	58 (48-67)	59 (48-68)	.46
Ever-smokers	59	60	.57
Smoking, pack-years†	17 (6-30)	15 (6-32)	.79
Alcohol, g/wk	96 (48-180)	96 (48-168)	.76
Number of children (women only)	2 (1-2)	2 (1-2)	.09
Nulliparous (women only)	13	17	.02
Age at first child, years (women only)	25 (22-29)	26 (23-29)	.56
Oral contraceptive‡	16	19	.36
Hormone replacement therapy§	16	16	.92
Low physical activity	41	39	.27
High intake of meat	46	44	.36
Low education	27	26	.66
Low income	14	13	.47
Family history of breast cancer‖	5	9	< .001
Family history of other cancers‖	23	24	.77
Body mass index, kg/m^2	26 (23-29)	26 (23-29)	.75
High C-reactive protein	18	17	.44

NOTE. Unless otherwise indicated, continuous variables are given as median (interquartile range), categorical variables given as percent.
*Wilcoxon rank sum or χ^2 test.
†Ever-smokers.
‡Premenopausal women.
§Postmenopausal women.
‖Number of first-degree relatives was from the Danish Civil Registration System, and their cancer diagnoses were from the national Danish Cancer Registry.
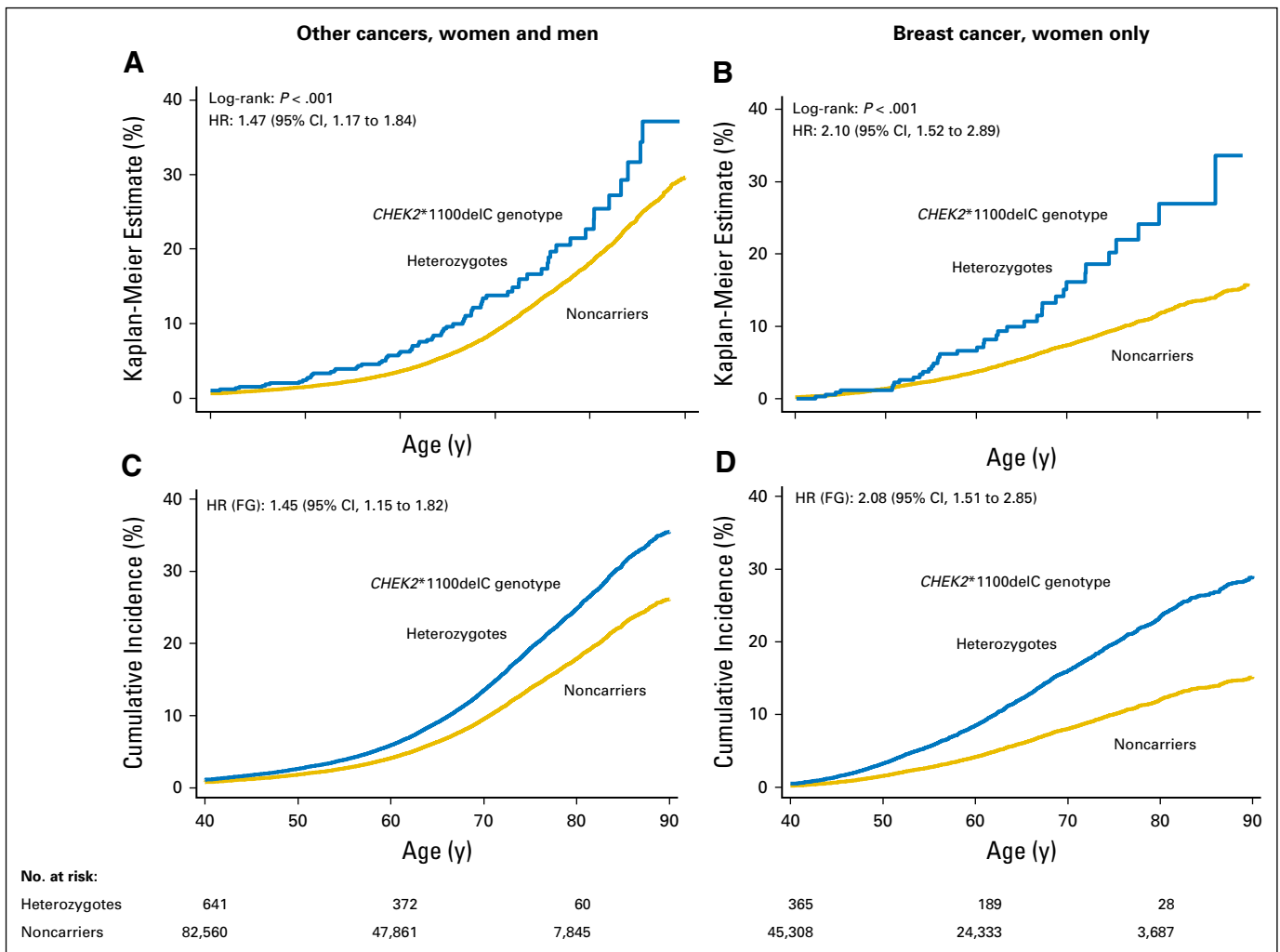
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Fig 1. Incidence of and risk for breast cancer and other cancers in *CHEK2**1100delC heterozygotes estimated from 86,975 individuals in the Copenhagen General Population Study. Kaplan-Meier estimates and risk for other cancers among women and men combined (A), and of breast cancer among women (B) according to genotype. The proportional hazard ratios were estimated using a Cox regression model adjusted for sex (only for other cancers) and age (as time scale). Kaplan-Meier estimates and risk for other cancers among women and men combined (C), and of breast cancer among women (D) according to genotype. The survival functions were estimated using Fine and Gray competing-risks regression with death and emigration as competing events, and were adjusted for sex (only for other cancers) and age (as time scale). HR, hazard ratio calculated in a Cox regression; HR (FG), hazard ratio calculated in a Fine and Gray regression model.

blood sampling, with no evidence of statistical sex difference after adjustment for 10 multiple tests (Fig 2). Hazard ratios for other cancers in *CHEK2**1100delC heterozygotes compared with noncarriers were 1.54 (95% CI, 1.08 to 2.18) for women and 1.37 (95% CI, 1.01 to 1.85) for men (sex difference *P* = .63). Importantly, the increased risk was not driven by nulliparous women, as the hazard ratio in parous women was 1.64 (95% CI, 1.13 to 2.38). Results from Cox proportional hazard regression models and multivariable adjustment were not different (data not shown).

Risk for Breast Cancer by Strata of Cancer Risk Factors

Risk for breast cancer for *CHEK2**1100delC heterozygotes was consistently increased, and not different when participants were stratified according to age, smoking status, alcohol consumption, nulliparity, family history of breast cancer and other cancers, body mass index, plasma C-reactive protein, and follow-up period before and after blood sampling, with no evidence of statistical

interaction after adjustment for nine multiple tests (Fig 3). Results from Cox proportional hazard regression models and multivariable adjustment were not different (data not shown).

Risks for Individual Cancer Types

For *CHEK2**1100delC heterozygotes compared with noncarriers, the age- and sex-adjusted hazard ratios were 5.76 (95% CI, 2.12 to 15.6) for stomach cancer, 3.61 (95% CI, 1.33 to 9.79) for kidney cancer, 3.45 (95% CI, 1.09 to 10.9) for sarcoma, and 1.60 (95% CI, 1.00 to 2.56) for prostate cancer (Fig 4). Results from Cox proportional hazard regression models or further multivariable adjustment were not different (data not shown). After taking 19 multiple tests into consideration, only risk for stomach cancer had a Bonferroni-adjusted *P* < .05. However, the distribution of the observed versus expected *P* values for the 19 individual cancer types suggested that *CHEK2**1100delC heterozygosity was associated with increased risk for at least one cancer (P < .001) (Fig 5).
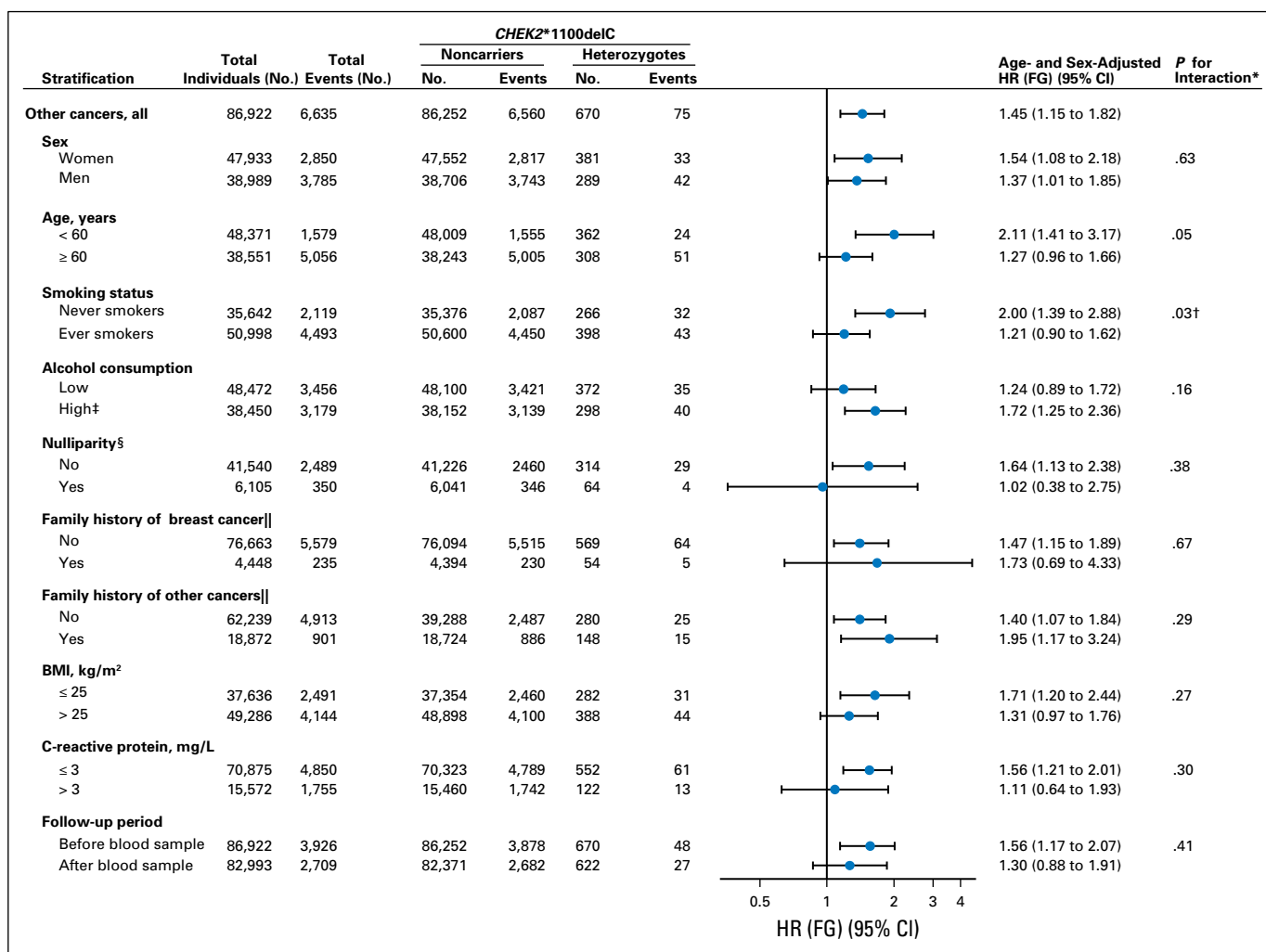
CHEK2*1100delC and Risk for Other Cancers

Stratification	Total Individuals (No.)	Total Events (No.)	CHEK2*1100delC Noncarriers No.	Noncarriers Events	Heterozygotes No.	Heterozygotes Events		Age- and Sex-Adjusted HR (FG) (95% CI)	P for Interaction*
Other cancers, all	86,922	6,635	86,252	6,560	670	75		1.45 (1.15 to 1.82)	
Sex									
Women	47,933	2,850	47,552	2,817	381	33		1.54 (1.08 to 2.18)	.63
Men	38,989	3,785	38,706	3,743	289	42		1.37 (1.01 to 1.85)	
Age, years									
< 60	48,371	1,579	48,009	1,555	362	24		2.11 (1.41 to 3.17)	.05
≥ 60	38,551	5,056	38,243	5,005	308	51		1.27 (0.96 to 1.66)	
Smoking status									
Never smokers	35,642	2,119	35,376	2,087	266	32		2.00 (1.39 to 2.88)	.03†
Ever smokers	50,998	4,493	50,600	4,450	398	43		1.21 (0.90 to 1.62)	
Alcohol consumption									
Low	48,472	3,456	48,100	3,421	372	35		1.24 (0.89 to 1.72)	.16
High‡	38,450	3,179	38,152	3,139	298	40		1.72 (1.25 to 2.36)	
Nulliparity§									
No	41,540	2,489	41,226	2460	314	29		1.64 (1.13 to 2.38)	.38
Yes	6,105	350	6,041	346	64	4		1.02 (0.38 to 2.75)	
Family history of breast cancer‖									
No	76,663	5,579	76,094	5,515	569	64		1.47 (1.15 to 1.89)	.67
Yes	4,448	235	4,394	230	54	5		1.73 (0.69 to 4.33)	
Family history of other cancers‖									
No	62,239	4,913	39,288	2,487	280	25		1.40 (1.07 to 1.84)	.29
Yes	18,872	901	18,724	886	148	15		1.95 (1.17 to 3.24)	
BMI, kg/m²									
≤ 25	37,636	2,491	37,354	2,460	282	31		1.71 (1.20 to 2.44)	.27
> 25	49,286	4,144	48,898	4,100	388	44		1.31 (0.97 to 1.76)	
C-reactive protein, mg/L									
≤ 3	70,875	4,850	70,323	4,789	552	61		1.56 (1.21 to 2.01)	.30
> 3	15,572	1,755	15,460	1,742	122	13		1.11 (0.64 to 1.93)	
Follow-up period									
Before blood sample	86,922	3,926	86,252	3,878	670	48		1.56 (1.17 to 2.07)	.41
After blood sample	82,993	2,709	82,371	2,682	622	27		1.30 (0.88 to 1.91)	

HR (FG) (95% CI) — 0.5 1 2 3 4

Fig 2. Risk for other cancers by strata of cancer risk factors in CHEK2*1100delC heterozygotes estimated from 86,975 individuals from the Copenhagen General Population Study. Fine and Gray proportional hazard ratios are given according to genotype for the risk for other cancers. Noncarriers was the reference group in the subgroup analyses stratified by sex, age, smoking status, alcohol consumption, nulliparity, family history of breast cancer and other cancers, body mass index (BMI), plasma C-reactive protein, and follow-up period before and after blood sampling. Hazard ratios were calculated in a Fine and Gray regression model and adjusted for age (as time scale) and sex; death and emigration were used as competing events. Numbers of participants for smoking status, nulliparity, family history of breast cancer and other cancers, and plasma C-reactive protein vary slightly because of incomplete information on a few participants. Follow-up period before blood sample was the period from the 20th year birthday until the time of blood sampling, and follow-up period after blood sample was the period from time of blood sampling until end of follow-up. *, according to Wald test; †, not significant after adjusting for 10 multiple tests according to the Bonferroni method (required P = .05/10 = .005); ‡, high consumption defined as more than168 g alcohol/wk for men and more than84 g alcohol/wk for women; §, only among women; ‖, number of first-degree relatives was from the Danish Civil Registration System, and their cancer diagnoses were from the national Danish Cancer Registry. Only individuals with one or more first-degree relatives (n = 81,162) were included in the particular analysis. HR (FG), hazard ratio calculated in a Fine and Gray regression model.

DISCUSSION

Estimated from 86,975 individuals in the Copenhagen General Population Study, of whom 6,635 developed other cancers than breast cancer, CHEK2*1100delC heterozygotes had a 15% to 82% increased risk for other cancers. This is a novel finding. It is of interest that risk for other cancers, by CHEK2*1100delC status, seemed to be slightly more pronounced in those younger than 60 years compared with those age 60 years and older.

Mechanistically considering the function of the CHEK2 protein, it is plausible that CHEK2*1100delC could be a susceptibility allele for any cancer, and not just for breast cancer. The CHEK2 protein is mainly activated by ataxia telangiectasia mutated protein in response to DNA double-strand breaks.[3] Subsequently, CHEK2 phosphorylates key cell cycle proteins, such as BRCA1, BRCA2, and P53, which, in turn, forces the cell to either apoptosis or to cell cycle arrest until the DNA is repaired.[5] Considering that all cells in CHEK2*1100delC heterozygous individuals lack half of intact CHEK2 proteins, it is conceivable that cells other than breast epithelial cells also could be sensitive to CHEK2 loss of function,[6] in accordance with the present observation of a 45% increased risk for other cancers in CHEK2*1100delC heterozygotes.

Previous studies have also investigated the risk for other cancer types than breast cancer, the majority reporting no significantly increased risk for CHEK2*1100delC heterozygotes compared with noncarriers for most cancer types.[7,15,20-23,25-31]

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Stratification	Total Individuals (No.)	Total Events (No.)	CHEK2*1100delC Noncarriers No.	CHEK2*1100delC Noncarriers Events	CHEK2*1100delC Heterozygotes No.	CHEK2*1100delC Heterozygotes Events		Age- and Sex-Adjusted HR (FG) (95% CI)	P for Interaction*
Breast cancer, women only	47,961	2,442	47,580	2,404	381	38		2.08 (1.51 to 2.85)	
Age, years									
< 60	27,177	762	26,941	750	229	12		1.88 (1.06 to 3.32)	.62
≥ 60	20,784	1,680	20,632	1,654	152	26		2.18 (1.49 to 3.19)	
Smoking status									
Never smokers	21,225	1,030	21,059	1,011	166	19		2.47 (1.58 to 3.85)	.32
Ever smokers	26,692	1,411	26,478	1,392	214	19		1.79 (1.14 to 2.82)	
Alcohol consumption									
Low	25,761	1,159	25,568	1,138	193	21		2.61 (1.69 to 4.03)	.16
High†	22,200	1,283	22,012	1,266	188	17		1.65 (1.04 to 2.64)	
Nulliparity‡									
No	41,565	2,131	41,251	2,098	314	33		2.18 (1.55 to 3.06)	.45
Yes	6,108	299	6,044	294	64	5		1.54 (0.65 to 3.64)	
Family history of breast cancer§									
No	42,339	2,015	42,032	1,988	307	27		1.99 (1.36 to 2.90)	.81
Yes	2,471	141	2,433	136	38	5		2.20 (1.02 to 4.76)	
Family history of other cancers§									
No	34,337	1,793	34,083	1,768	254	25		2.05 (1.39 to 3.04)	.85
Yes	10,473	363	10,382	356	91	7		2.11 (1.06 to 4.60)	
BMI, kg/m²									
≤ 25	24,915	1,193	24,717	1,176	198	17		1.86 (1.15 to 3.00)	.52
> 25	23,046	1,249	22,863	1,228	183	21		2.30 (1.50 to 3.51)	
C-reactive protein, mg/L									
≤ 3	38,627	1,919	38,309	1,888	318	31		1.99 (1.40 to 2.82)	.54
> 3	9,069	516	9,008	509	61	7		2.55 (1.22 to 5.34)	
Follow-up period									
Before blood sample	46,194	1,767	45,836	1,744	358	23		1.72 (1.14 to 2.59)	.11
After blood sample	42,834	675	42,504	660	330	15		3.02 (1.80 to 5.05)	

Hazard Ratio (FG) (95% CI)
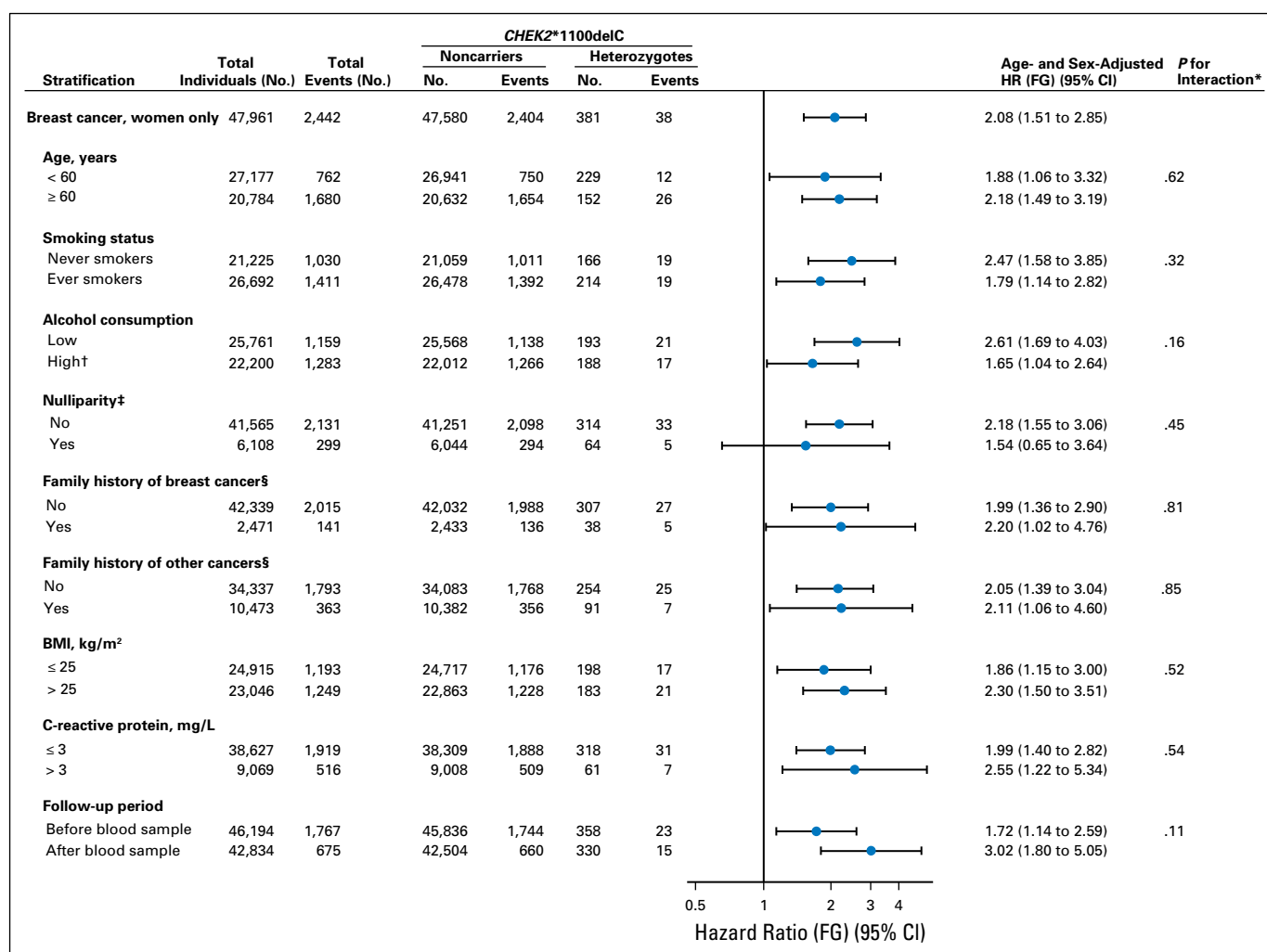0.5 1 2 3 4

Fig 3. Risk for breast cancer by strata of cancer risk factors in CHEK2*1100delC heterozygotes estimated from 47,961 women from the Copenhagen General Population Study. Fine and Gray proportional hazard ratios are given according to genotype for the risk of breast cancer. "Noncarriers" was the reference group in the subgroup analyses stratified on age, smoking status, alcohol consumption, nulliparity, family history of breast cancer and other cancers, body mass index (BMI), plasma C-reactive protein, and follow-up period before and after blood sampling. Hazard ratios were calculated in a Fine and Gray regression model and adjusted for age (as time scale) and death and emigration were used as competing events. Numbers of participants for smoking status, nulliparity, family history of breast cancer and other cancers, and plasma C-reactive protein vary slightly due to incomplete information on a few participants. Follow-up period before blood sample was the period from the 20th year birthday until the time of blood sampling, and follow-up period after blood sampling was the period from time of blood sampling until end of follow-up. *, according to Wald test; †, high consumption defined as more than 84 g alcohol/wk; ‡, only among women; §, number of first-degree relatives was from the Danish Civil Registration System, and their cancer diagnoses were from the national Danish Cancer Registry. Only individuals with one or more first-degree relatives were included in the particular analysis (n = 44,810). HR (FG), hazard ratio calculated in a Fine and Gray regression model.

These studies, as in our own, had lower power to detect risk for individual cancer types because of the rarity of the risk allele, as exemplified by the broad confidence interval (0.43 to 1.72) for our hazard estimate for colon cancer. However, the overall distribution of P values of risks for the individual cancers suggested that CHEK2*1100delC heterozygosity is associated with increased risk for at least one cancer type, and the combined end point of other cancers provided sufficient power in this study to detect that CHEK2*1100delC heterozygosity is associated with increased risk for other types of cancer than breast cancer.

CHEK2*1100delC heterozygosity is now an established breast cancer susceptibility allele.[7,9-14] Because of the frequency of CHEK2*1100delC of 1.2% (95% CI, 0.7% to 1.8%) in the Netherlands[15] and its penetrance at age 70 for breast cancer of 37% to 59%,[12,42] comparable to the corresponding penetrance at age 70

of 41% to 75% for BRCA1 and BRCA2 mutations,[12,43,44] a routine test for this mutation has been offered since September 2014, together with BRCA1 and BRCA2 sequencing of women with familial breast cancer[17] in the Netherlands.[19] To the best of our knowledge, no other country has yet introduced screening of CHEK2*1100delC together with BRCA1 and BRCA2. However, the presumed clinical benefit of identifying CHEK2*1100delC carriers by offering them surveillance programs to detect breast cancer at an early stage,[19] in combination with the ease and low cost of the test, will probably lead to increased clinical use of genotyping for CHEK2*1100delC in the future. Although CHEK2*1100delC heterozygosity is associated with a two- to three-fold increased risk for breast cancer in the general population at a median age of 53 years,[7] currently there is a lack of data on corresponding risk for cancers at old ages.

	Total Individuals (No.)	Total Events (No.)	CHEK2*1100delC Noncarriers No.	Noncarriers Events	Heterozygotes No.	Heterozygotes Events		Age- and Sex-Adjusted HR (FG) (95% CI)	P
Breast cancer	47,961	2,442	47,580	2,404	381	38		2.08 (1.51 to 2.85)	< .001
Other cancers, all	86,922	6,635	86,252	6,560	670	75		1.45 (1.15 to 1.82)	.002
Stomach cancer	86,975	89	86,305	85	670	4		5.76 (2.12 to 15.6)	.001
Kidney cancer	86,974	138	86,304	134	670	4		3.61 (1.33 to 9.79)	.01*
Sarcoma	86,966	110	86,296	107	670	3		3.45 (1.09 to 10.9)	.04*
Prostate cancer	39,014	1,340	38,725	1,322	289	18		1.60 (1.00 to 2.56)	.05
Malignant melanoma	86,972	898	86,302	886	670	12		1.70 (0.96 to 3.00)	.07
Bladder cancer	86,975	272	86,305	267	670	5		2.26 (0.94 to 5.43)	.07
Ovary cancer	47,961	231	47,580	227	381	4		2.23 (0.83 to 5.99)	.11
Hodgkin lymphoma	86,974	27	86,304	26	670	1		4.74 (0.64 to 35.1)	.13
Multiple myeloma	86,975	100	86,305	98	670	2		2.46 (0.61 to 9.97)	.21
Uterus cancer	47,960	386	47,579	381	381	5		1.70 (0.70 to 4.14)	.24
Brain cancer	86,957	128	86,287	126	670	2		1.96 (0.49 to 7.92)	.35
Leukemia	86,972	218	86,302	217	670	1		0.56 (0.08 to 4.01)	.49
Cervix cancer	47,961	252	47,580	251	381	1		0.50 (0.07 to 3.60)	.56
Colon cancer	86,975	1,131	86,305	1,123	670	8		0.86 (0.43 to 1.72)	.68
Lung cancer	86,974	509	86,304	504	670	1		1.20 (0.49 to 2.90)	.69
Oral cancer	86,973	194	86,303	192	670	2		1.27 (0.31 to 5.16)	.73
Testis cancer	39,011	209	38,722	207	289	2		1.27 (0.32 to 5.13)	.73
Thyroid cancer	86,973	100	86,303	99	670	5		1.27 (0.18 to 9.09)	.81

Hazard Ratio (FG) (95% CI): 0.2 0.6 1 2 4 6 10

Fig 4. Risk of individual cancer types in CHEK2*1100delC heterozygotes estimated from 86,975 individuals from the Copenhagen General Population Study. Fine and Gray proportional hazard ratios are given according to genotype and by individual cancer types, and are listed from lowest to highest P value. "Noncarriers" was the reference group. Hazard ratios were age (as time scale) and sex-adjusted (not in female and male cancers), and death and emigration were used as competing events. Total number of participants varies slightly because of varying numbers of excluded participants; that is, participants with the individual cancer type in question before start of follow-up. Only cancer types with events among heterozygotes were shown; we found no heterozygotes with laryngeal cancer, pancreatic cancer, liver cancer, non-Hodgkin's lymphoma, or esophageal cancer; therefore, we could not estimate risk for these cancers. *, not statistically significant after adjusting for 19 multiple tests according to the Bonferroni method (required P = .05/19 = .003). HR (FG), hazard ratio calculated in a Fine and Gray regression model.

Women with estrogen receptor-positive breast cancer with CHEK2*1100delC have poorer survival,[13] and this prognostic information might be translated into altered clinical management of these patients. Therefore, with the increasing number of persons tested for this mutation in the Netherlands and elsewhere, there is an increasing need for evidence regarding other morbidities for CHEK2*1100delC heterozygotes, as provided in the current study, to perform genetic counseling before and after testing.

We found a carrier frequency of CHEK2*1100delC of 0.80% (95% CI, 0.71% to 0.83%) in individuals in the Danish general population, similar to what has been observed previously.[7,9] Furthermore, our risk estimates for breast cancer were in agreement with two available meta-analyses,[12,14] supporting the validity of our genotyping and ascertainment of cancer development.

Other strengths of the current study include the large sample size of 86,975 individuals and the comprehensive survey for cancer diagnoses from 1943 through 2011 from the Danish Cancer Registry, capturing 98% of all cancers in Denmark.[33,34] In this registry, all cancer diagnoses are confirmed with pathologic

findings. Finally, we used identical genotyping for all participants, thereby minimizing the possibility for bias in genotyping.

Some limitations to our study must be considered. First, the number of heterozygous individuals with each individual cancer type was small, so we had limited power. Second, despite the fact that the genotype distribution did not differ from Hardy-Weinberg equilibrium and our risk estimates for breast cancer are similar to previous reports, we cannot totally exclude that CHEK2*1100delC heterozygotes were less likely to participate than noncarriers. Such potential survival bias is conservative, and if it has influenced our risk estimates, would likely only tend to bias our results toward the null and, thus, cannot explain our results for other cancers. Finally, as we studied white people of Northern European descent only, our results may not necessarily apply to all countries and ethnic groups; however, we are not aware of data suggesting that the present results should not apply to most countries where the CHEK2*1100delC mutation is found; that is, in populations originating from northern and western Europe.[11,13]
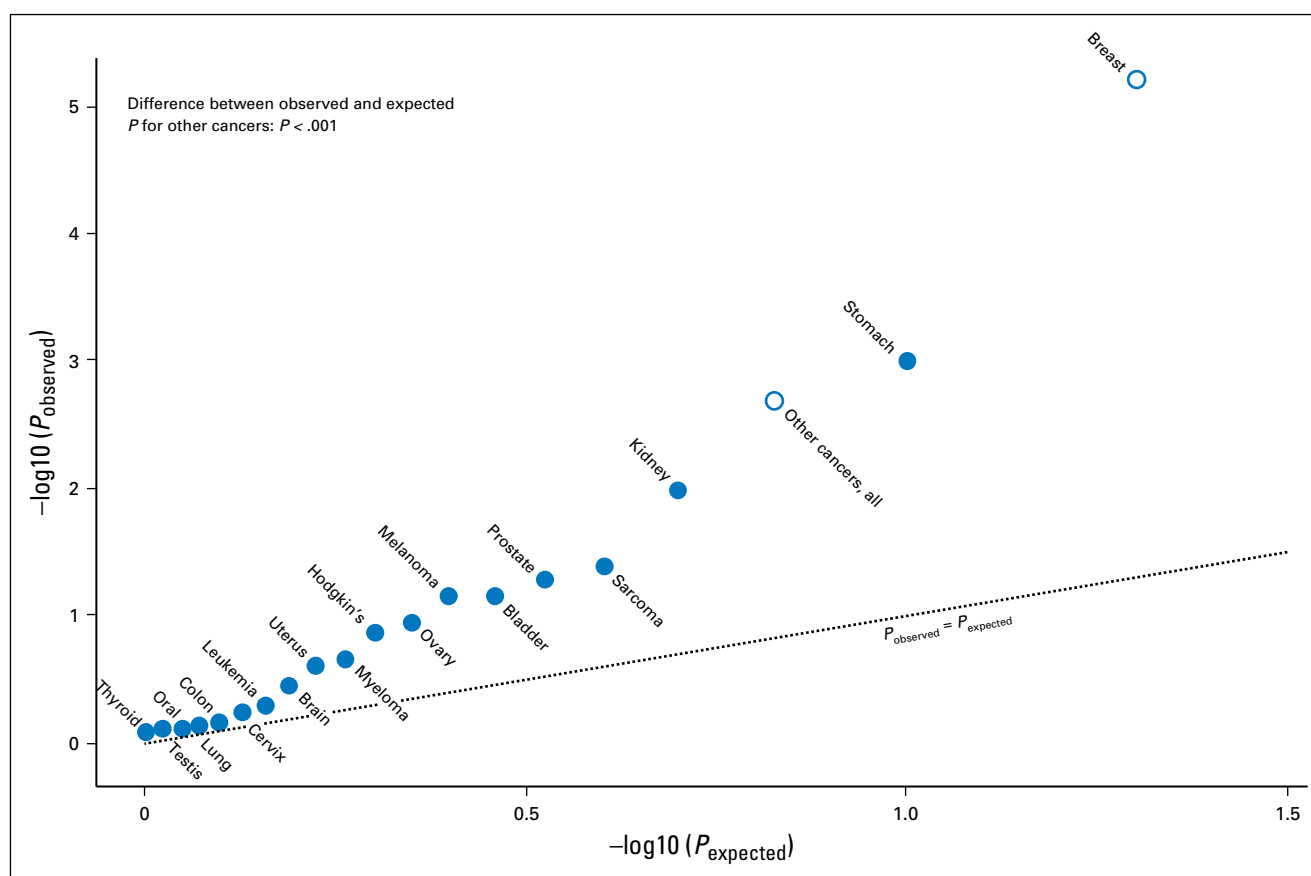
Näslund-Koch, Nordestgaard, and Bojesen

Fig 5. Observed versus expected P values for risk for cancer types in *CHEK2**1100delC heterozygotes estimated from 86,975 individuals from the Copenhagen General Population Study. The observed P values from Figure 4 were plotted against the expected P values under the null hypothesis that *CHEK2**1100delC heterozygosity was not associated with risk for any individual cancer type. P values reflect significance against the uniform distribution (χ^2 distribution with 38 degrees of freedom). Open circles = a priori hypothesized cancers; closed circles = cancers included in the explorative analyses.

In conclusion, our findings support the hypothesis that in the general population, *CHEK2**1100delC heterozygosity is associated with an increased risk for at least some cancers in addition to breast cancer. This information may be useful in clinical counseling of patients with this loss-of-function mutation.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at www.jco.org.

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REFERENCES

1. Ferlay J, Soerjomataram I, Dikshit R, et al: Cancer incidence and mortality worldwide: Sources, methods and major patterns in GLOBOCAN 2012. Int J Cancer 136:E359-E386, 2015

2. Yao Y, Dai W: Genomic instability and cancer. J Carcinog Mutagen 5:1000165, 2014

3. Hirao A, Kong YY, Matsuoka S, et al: DNA damage-induced activation of p53 by the checkpoint kinase Chk2. Science 287:1824-1827, 2000

4. Bartek J, Falck J, Lukas J: CHK2 kinase–A busy messenger. Nat Rev Mol Cell Biol 2:877-886, 2001

5. Cai Z, Chehab NH, Pavletich NP: Structure and activation mechanism of the CHK2 DNA damage checkpoint kinase. Mol Cell 35:818-829, 2009

6. Bartek J, Lukas J: Chk1 and Chk2 kinases in checkpoint control and cancer. Cancer Cell 3:421-429, 2003

7. Weischer M, Bojesen SE, Tybjaerg-Hansen A, et al: Increased risk of breast cancer associated with CHEK2*1100delC. J Clin Oncol 25:57-63, 2007

8. Bell DW, Varley JM, Szydlo TE, et al: Heterozygous germ line hCHK2 mutations in Li-Fraumeni syndrome. Science 286:2528-2531, 1999

9. Meijers-Heijboer H, van den Ouweland A, Klijn J, et al; CHEK2-Breast Cancer Consortium: Low-penetrance susceptibility to breast cancer due to CHEK2(*)1100delC in noncarriers of BRCA1 or BRCA2 mutations. Nat Genet 31:55-59, 2002

10. Vahteristo P, Bartkova J, Eerola H, et al: A CHEK2 genetic variant contributing to a substantial fraction of familial breast cancer. Am J Hum Genet 71:432-438, 2002

11. CHEK2 Breast Cancer Case-Control Consortium: CHEK2*1100delC and susceptibility to breast cancer: A collaborative analysis involving 10,860 breast cancer cases and 9,065 controls from 10 studies. Am J Hum Genet 74:1175-1182, 2004

12. Weischer M, Bojesen SE, Ellervik C, et al: CHEK2*1100delC genotyping for clinical assessment of breast cancer risk: Meta-analyses of 26,000 patient cases and 27,000 controls. J Clin Oncol 26:542-548, 2008

13. Weischer M, Nordestgaard BG, Pharoah P, et al: CHEK2*1100delC heterozygosity in women with breast cancer associated with early death, breast cancer-specific death, and increased risk of a second breast cancer. J Clin Oncol 30:4308-4316, 2012

14. Yang Y, Zhang F, Wang Y, et al: CHEK2 1100delC variant and breast cancer risk in Caucasians: A meta-analysis based on 25 studies with 29,154 cases and 37,064 controls. Asian Pac J Cancer Prev 13:3501-3505, 2012

15. Huijts PE, Hollestelle A, Balliu B, et al: CHEK2*1100delC homozygosity in the Netherlands–Prevalence and risk of breast and lung cancer. Eur J Hum Genet 22:46-51, 2014

16. Adank MA, Verhoef S, Oldenburg RA, et al: Excess breast cancer risk in first degree relatives of CHEK2∗1100delC positive familial breast cancer cases. Eur J Cancer 49:1993-1999, 2013

17. Byrnes GB, Southey MC, Hopper JL: Are the so-called low penetrance breast cancer genes, ATM, BRIP1, PALB2 and CHEK2, high risk for women with strong family histories? Breast Cancer Res 10:208, 2008

18. Cybulski C, Wokołorczyk D, Jakubowska A, et al: Risk of breast cancer in women with a CHEK2 mutation with and without a family history of breast cancer. J Clin Oncol 29:3747-3752, 2011

19. Adank MA, Hes FJ, van Zelst-Stams WAG, et al: CHEK2-mutatie in Nederlandse borstkankerfamilies: Uitbreiding van de genetische diagnostiek op borstkanker [in Dutch]. Ned Tijdschr Geneeskd 159: A8910, 2015

20. Meijers-Heijboer H, Wijnen J, Vasen H, et al: The CHEK2 1100delC mutation identifies families with a hereditary breast and colorectal cancer phenotype. Am J Hum Genet 72:1308-1314, 2003

21. Seppälä EH, Ikonen T, Mononen N, et al: CHEK2 variants associate with hereditary prostate cancer. Br J Cancer 89:1966-1970, 2003

22. Cybulski C, Huzarski T, Górski B, et al: A novel founder CHEK2 mutation is associated with increased prostate cancer risk. Cancer Res 64: 2677-2679, 2004

23. Cybulski C, Górski B, Huzarski T, et al: CHEK2 is a multiorgan cancer susceptibility gene. Am J Hum Genet 75:1131-1135, 2004

24. Thompson D, Seal S, Schutte M, et al: A multicenter study of cancer incidence in CHEK2 1100delC mutation carriers. Cancer Epidemiol Biomarkers Prev 15:2542-2545, 2006

25. Xiang HP, Geng XP, Ge WW, et al: Meta-analysis of CHEK2 1100delC variant and colorectal cancer susceptibility. Eur J Cancer 47:2546-2551, 2011

26. Weischer M, Heerfordt IM, Bojesen SE, et al: CHEK2*1100delC mutation and risk of malignant melanoma: Danish and German studies and meta-analysis. J Invest Dermatol 132:299-303, 2012

27. Hale V, Weischer M, Park JY: CHEK2 (∗) 1100delC mutation and risk of prostate cancer. Prostate Cancer 2014:294575, 2014

28. Koppert LB, Schutte M, Abbou M, et al: The CHEK2(*)1100delC mutation has no major contribution in oesophageal carcinogenesis. Br J Cancer 90:888-891, 2004

29. Sellick GS, Sullivan K, Catovsky D, et al: CHEK2*1100delC and risk of chronic lymphocytic leukemia. Leuk Lymphoma 47:2659-2660, 2006

30. Krylova NY, Ponomariova DN, Sherina NY, et al: CHEK2 1100 delC mutation in Russian ovarian cancer patients. Hered Cancer Clin Pract 5:153-156, 2007

31. Siołek M, Cybulski C, Gąsior-Perczak D, et al: CHEK2 mutations and the risk of papillary thyroid cancer. Int J Cancer 137:548-552, 2015

32. Jørgensen AB, Frikke-Schmidt R, Nordestgaard BG, et al: Loss-of-function mutations in APOC3 and risk of ischemic vascular disease. N Engl J Med 371:32-41, 2014

33. Storm HH: Completeness of cancer registration in Denmark 1943-1966 and efficacy of record linkage procedures. Int J Epidemiol 17:44-49, 1988

34. Storm HH, Michelsen EV, Clemmensen IH, et al: The Danish Cancer Registry–History, content, quality and use. Dan Med Bull 44:535-539, 1997

35. World Health Organization. Third Report of the Expert Committee on Health Statistics. Geneva, Switzerland World Health Organization, 1952

36. Bray F, Sankila R, Ferlay J, et al: Estimates of cancer incidence and mortality in Europe in 1995. Eur J Cancer 38:99-166, 2002

37. Allin KH, Bojesen SE, Nordestgaard BG: Baseline C-reactive protein is associated with incident cancer and survival in patients with cancer. J Clin Oncol 27:2217-2224, 2009

38. Allin KH, Nordestgaard BG, Zacho J, et al: C-reactive protein and the risk of cancer: A Mendelian randomization study. J Natl Cancer Inst 102:202-206, 2010

39. Scherczinger CA, Bourke MT, Ladd C, et al: DNA extraction from liquid blood using QIAamp. J Forensic Sci 42:893-896, 1997

40. Fine J, Gray R: A proportional hazards model for the subdistribution of a competing risk. J Am Stat Assoc 94:496-509, 1999

41. Odefrey F, Stone J, Gurrin LC, et al; Australian Twins and Sisters Mammographic Density Study: Common genetic variants associated with breast cancer and mammographic density measures that predict disease. Cancer Res 70:1449-1458, 2010

42. Johnson N, Fletcher O, Naceur-Lombardelli C, et al: Interaction between CHEK2*1100delC and other low-penetrance breast-cancer susceptibility genes: A familial study. Lancet 366:1554-1557, 2005

43. Chen S, Parmigiani G: Meta-analysis of BRCA1 and BRCA2 penetrance. J Clin Oncol 25:1329-1333, 2007

44. Mavaddat N, Peock S, Frost D, et al: EMBRACE: Cancer risks for BRCA1 and BRCA2 mutation carriers: Results from prospective analysis of EMBRACE. J Natl Cancer Inst 105:812-822, 2013

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Increased Risk for Other Cancers in Addition to Breast Cancer for *CHEK2**1100delC Heterozygotes Estimated From the Copenhagen General Population Study

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